FURTHER STUDIES ON FUSIFORM BACILLI AND SPIRILLA.*

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In a previous article[1] Dr. Weaver and I described three strains of fusiform bacilli which were isolated in pure culture from cases of ulceromembranous angina, ulceromembranous stomatitis, and diphtheria of the tonsil. These organisms were anaerobic and appeared to be the same as those described by Ellermann.[2]

In a later communication I[3] described three strains of fusiform bacilli isolated in pure culture from the normal mouth. These organisms were anaerobic and differed only slightly culturally from those described by Dr. Weaver and me. Morphologically I found that after growing a few days filaments and decidedly spiral forms appeared. The spiral forms resembled the spirilla found in the smears made from the mouth and found in conjunction with the fusiform bacillus in ulceromembranous angina (Vincent's angina), ulceromembranous gingivitis, and noma. This was in accord with the opinion of various observers (Seiffert,[4] Perthes,[5] Herrman,[6] and Neuof[7]) that the fusiform bacilli and spirilla are different forms of one organism. Several of these authors describe it as a cladothrix or streptothrix. Seiffert isolated in pure culture a cladothrix from two cases of noma. This organism produced threads, "spirilla," and branching forms of cladothrix. No branching could be seen in any of my cultures.

Ghon and Mucha[8] and Kaspar and Kern[9] isolated from abscesses anaerobic fusiform bacilli, which formed long filaments and were also extremely polymorphous.

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[1] *Jour. Infect. Dis.*, 1905, 2, p. 446.

[2] *Centralbl. f. Bakt.*, Abt. 1, Orig., 1904, 37, p. 729.

[3] *Jour. Infect. Dis.*, 1906, 3, p. 148.

[4] *Münch. Med. Wchnschr.*, 1901, 49, p. 1988.

[5] *Archiv. f. klin. Chir.*, 1899, 59, p. 111.

[6] *Archiv. f. Pediatrics*, 1905, 22, p. 817.

[7] *Amer. Jour. Med. Sci.*, 1910, 139, p. 705.

[8] *Centralbl. f. Bakt.*, Abt. 1, Orig., 1909, 49, p. 493.

[9] *Ibid.*, 1910, 55, p. 7.

Since isolating the fusiform bacilli and spirilla from the normal mouth, I have isolated in pure culture three strains from cases of ulceromembranous angina (Vincent's angina), one from a case of ulceromembranous gingivitis, and one from a case of noma of the face.[1]

The organisms from the cases of Vincent's angina were isolated on slants of ascites-agar (1:3), those from the cases of gingivitis and noma on blood-agar. The material was smeared on a series of slants. Usually in the second tube the colonies of fusiform bacilli could be found after three or four days' incubation at 37° C. The cultures were grown anaerobically according to the method of Wright, by saturating the cotton stopper with a strong solution of pyrogallic acid in a five per cent solution of sodium hydroxide, and closing the tube with a tightly fitting cork and sealing it with paraffin. The sub-cultures were made from single colonies.

The colonies are delicate and whitish and resemble colonies of streptococci, but are rather smoother and softer, especially when grown on blood-agar. When the colonies are quite isolated, they sometimes attain considerable size (0.3 cm. in diameter). The organisms are obligate anaerobes growing at 37° C. The cultures from the normal mouth grew slightly at room temperature and aerobically on dog's blood-agar after long isolation. The organisms show no progressive but considerable vibratory motion, the spirilla retaining their spiral form. They grow on ascites-agar, Loeffler's blood-serum, blood-agar (dog, sheep, goat, rabbit, and human), and horse and human serum-agar. The best spirilla are most frequently found in a medium of agar (5 c.c.) and 12 drops of equal parts of goat's or sheep's blood and two per cent sodium citrate solution in normal salt solution. An offensive odor is given off in all successful cultures. As the organisms die out rapidly it is necessary to transplant a large number of organisms to get a successful culture.

The organisms are extremely polymorphous. They are usually during the first 24 hours' growth delicate pointed rods 3–10 mikrons in length, straight or slightly bent. Spores are often seen during

[1] I am indebted to Dr. Thomas L. Gilmer for the opportunity of making cultures from the case of gingivitis and to Dr. Boughton and Dr. Hayhurst for assistance in obtaining material from the case of noma.

the first days of growth. Filaments of various lengths may appear in 24–48 hours or later. They are found most often in cultures on Loeffler's blood-serum. As most of these cultures were made on blood-agar the usual absence of filaments may be accounted for. When filaments are present they can often be seen to be made up of strings of bacilli. They frequently contain deeply staining bodies, sometimes round, more often like bands. Vacuoles are sometimes seen in the filaments.

Occasionally on the first day of growth, but usually a few days later, spirals with from one to twenty curves are observed, sometimes in large numbers. As a rule they stain uniformly, but often it can be seen that they too are made up of rods. The spirilla sometimes contain vacuoles similar to those seen in the spirilla in smears made from the tissues of Vincent's angina and noma. On the sodium citrate and blood-agar and human serum-agar the spirilla appear shorter and more curved than on the other media employed. Involution forms in a great variety of shapes are seen in some of the older cultures.

Sometimes for several generations fusiform bacilli only will be found in the cultures, but on changing the medium, filaments and spirilla will again be formed.

The organisms stain best with heated one per cent solution of alcoholic gentian-violet in five per cent carbolic acid solution. The spirilla appear thicker when so stained than the spirilla seen in smears from lesions which have been first fixed with heat and then stained. However, when such specimens are stained deeply by the above method, the spirilla often appear just as thick as those artificially cultivated. The spirilla may also be seen to good advantage in India ink preparations. The organisms do not stain by Gram's method.

Animal experiments have been unsuccessful. Large numbers of bacteria were injected, the growth on four and five slants being used. Injections were made subcutaneously, intraperitoneally, intravenously, and into the mucous membrane of the mouth. The animals experimented with were the dog, guinea-pig, rabbit, white rat, and pigeon.

Efforts to first lower the resistance of the animal by injections

of bichloride of mercury, lactic acid, and large numbers of killed streptococci were without any effect.

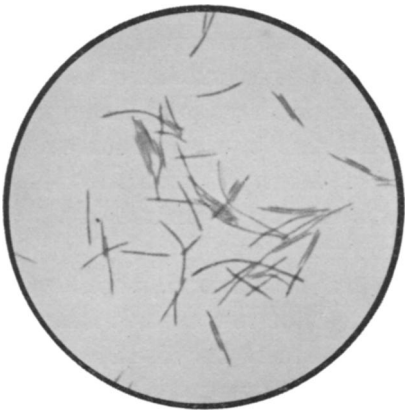

FIG. 1.—Pure culture of fusiform bacilli from noma, grown 48 hours anaerobically on goat's blood-agar. Carbol-gentian-violet. ×1,000.

FIG. 2.—Pure culture of fusiform bacilli from noma, grown anaerobically on goat's blood-agar. Carbol-gentian-violet. ×750.

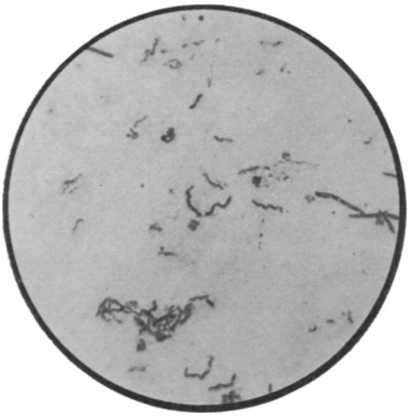

FIG. 3.—Pure culture of fusiform bacilli from noma, grown 10 days anaerobically on human serum-agar. Carbol-gentian-violet. ×1,000.

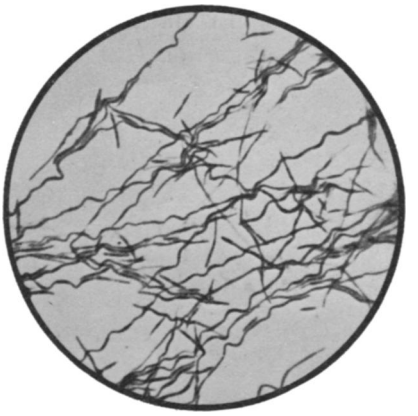

FIG. 4.—Pure culture of fusiform bacilli from the normal mouth, grown 48 hours anaerobically in 0.25 per cent glucose broth. Carbol-gentian-violet. ×1,000.

Sterile animal tissues from the dog and guinea-pig were also inoculated, but no growth of the organism occurred. It is possible that the length of time elapsing before the growth of the organisms (3–5 days) and the time required to isolate them in pure culture, generally several weeks, may account for their lack of virulency.

The case of noma from which a pure culture of fusiform bacilli and spirilla was isolated developed in a man 50 years old. The necrosis started in an ulcer on the cheek adjacent to two irregular teeth. When the patient was first seen there was great swelling and induration of the cheek, which had been perforated by the attending surgeon. There was considerable discharge from the opening in the cheek. The mucous membrane of the mouth was sloughing off and the odor was very offensive. Fusiform bacilli, filaments, and spirilla were the predominating organisms in the smears made from the gangrenous material.

The patient's opsonic index was 0.4 to a fusiform bacillus isolated from a case of gingivitis. On account of the low opsonic index, subcutaneous injections were made of fusiform bacilli, killed by heating for an hour at 60° C. These organisms were taken up quite freely by the leukocytes without the presence of serum, but not to such a degree as to interfere greatly with the estimation of the opsonic index. This strain differed in this respect from those previously described,[1] which were as readily phagocytable without as with serum, possibly because of long cultivation outside of the body before being tested.

The injections of killed fusiform bacilli and spirilla did not cause any local reaction at the site of inoculation. Following each injection there was an increase in the amount of opsonin as shown in the chart. At one time there seemed to be considerable improvement in the face, much of the swelling and induration disappearing, but it now developed that a carcinoma of the face had started, from which the patient soon died.

The cheek tissue removed during life showed carcinomatous tissue adjacent but distinct from the gangrenous tissue. This showed complete necrosis of the superficial skin with a line of demarcation separating it from the living tissue. In the superficial necrotic tissue were found cocci, fusiform bacilli, filaments, and spirilla, the last three organisms predominating. In the region of leukocytic invasion the fusiform bacilli, filaments, and spirilla alone were observed. A thick network of filaments, some being wavy, was seen outside the line of demarcation. The organisms

[1] *Jour. Infect. Dis.*, 1907, 4, p. 66.

could not be found invading the healthy tissue. The pure cultures of fusiform bacilli and spirilla isolated from this patient closely

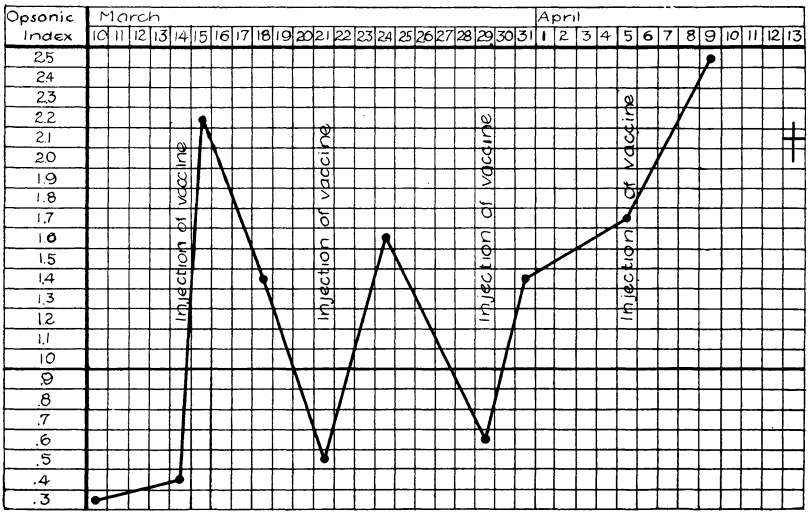

CHART 1.—Opsonic curve in case of noma injected with killed culture of fusiform bacilli and spirilla.

March 15.—Growth on one blood-agar slant injected subcutaneously.
March 21.—Growth on one and one-half blood-agar slants injected subcutaneously.
March 29.—Growth on two blood-agar slants injected subcutaneously.
April 5.—Growth on one and one-half blood-agar slants injected subcutaneously.

resembled the organisms in the tissues and in smear preparations from the necrotic tissue.

CONCLUSIONS.

The strains of fusiform bacilli isolated in pure culture from the normal mouth, ulceromembranous angina and gingivitis, and noma appear culturally and morphologically to be the same organism. The cultures show that the bacilli and the spirilla are different forms of one organism. Whether the spirilla formed from the fusiform bacilli are the same as those found in the lesions themselves cannot be decided on account of the inability to reproduce the lesions in lower animals. The injection of killed cultures of fusiform bacilli and spirilla in the case of noma caused an increase in the amount of specific opsonin and appeared to produce some beneficial effect.